Crystal Structure of Outer Surface Protein C (OspC) from the Lyme Disease Spirochete, *Borrelia burgdorferi*

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The three-dimensional structure of Outer surface protein C (OspC), a major antigen on the surface of the Lyme disease spirochete, *Borrelia burgdorferi*, when it is being transmitted to humans, has been determined to 1.8 Å resolution. The structure is predominantly helical. This is in contrast to the structure of OspA, a major surface protein mainly present when spirochetes are residing in the midgut of unfed ticks, which is mostly β sheet. The surface of OspC that would project away from the spirochete's membrane has a region of strong negative electrostatic potential that may be involved in binding to positively charged host ligands. This feature is present only on OspCs from strains known to cause invasive human disease.

Cloning, expression and purification procedures are described elsewhere (Dunn *et al*. in preparation). Crystals of the SeMet OspC protein (aa 38-201) were by vapor diffusion in sitting drops at 293K with a precipitant containing 25% (v/v) PEG monomethyl ether 550, 10 mM zinc sulfate heptahydrate, 100 mM MES pH 6.5. Diffraction data extending to 2.8 Å were collected from the frozen crystal with the B1 detector on beamline X12C at the NSLS. Multiwavelength Anomalous Diffraction (MAD) data were collected around the selenium absorption edge. The data were processed with DENZO and SCALEPACK. Subsequently, single wavelength high-resolution data extending to 1.8 Å were collected with a better quality SeMet OspC crystal (Form II) at X25 beamline at the NSLS and were used in the later stages of refinement.

There are four molecules per asymmetric unit and each molecule has two selenomethionines. The selenium positions were obtained from Patterson and difference Fourier maps with the use of the PHASES program package. There are two dimers in the asymmetric unit. The monomers are related by a non-crystallographic two-fold axis forming a dimer and the dimers are related by a pseudo translation of half the unit cell along the c axis. A total of 8 selenium atoms were input to the SHARP program to refine the phases. The resulting phases were further improved by NCS averaging in DM in two steps, averaging the non-crystallographic two-fold related monomers first and then the dimers related by pseudo translational symmetry. The resulting electron density map was of excellent quality and revealed an almost all-helical structure. The model building was completed using the two selenomethionines as markers. Further refinement was carried out with the high-resolution data set. Model was refined by the slow cool annealing method alternating with model building until convergence.

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